On the Possible Significance of Enhanced Glutamate Dehydrogenase Activity in Normal and Aestivated *Pila globosa*

BY

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(3 Text figures)

INTRODUCTION

Active life of the snail, *Pila globosa* (Swainson, 1822), is interrupted at intervals, during drought conditions, by aestivation during which the animal enters into a state of metabolic dormancy (Meenakshi, 1956a; Garten, 1958; Raghupathirami Reddy, 1965). During aestivation there is a decrease in the activities of Krebs cycle enzymes and the animal survives by anaerobic glycolysis utilizing slowly the glycogen reserve (Slater, 1928; von Brand, Baernstein & Mehlman, 1950; Mehlman & von Brand, 1951, 1953; von Brand, McMahon & Nolan, 1955; Meenakshi, 1956a, 1957; and Reddy, *op. cit.*). The glutamate level was found to increase in the soft parts of the aestivating snail, which was suggested to be of significance during aestivation (Murali Mohan, 1973; Murali Mohan *et al.*, 1973; Ramana Rao, 1973). However, very little information is available regarding its specific involvement in the aestivation metabolism of *Pila globosa*. Janssens (1964) pointed out that "during aestivation there would be changes in the intermediary metabolism of great interest to the comparative biochemist but these have as yet been the subject of little experimental investigation." An attempt is made here to study the activity pattern of glutamate dehydrogenase during aestivation.

MATERIALS AND METHODS

Pila globosa were collected from the local fresh-water ponds and were maintained in aquaria for a week by feeding them on *Hydrilla* plants. Actively feeding snails were aestivated by embedding them in dry sand for different periods as required.

A 10% (w/v) homogenate of the hepatopancreas of normal and aestivated snails were prepared in 0.25 M sucrose in a chilled glass homogenizer. The homogenates were centrifuged at 4000 rpm and the supernatant was assayed for the enzyme activity.

The glutamate dehydrogenase (EC 1.4.1.3) was studied by the dye-reduction method (Srikanthan & Krishnamurthy, 1955) as modified by Govindappa & Swami (1965) using 2-(p-Idophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) (Koch-Light Laboratories Ltd., England) as the terminal electron acceptor. The assay mixture consisted of 50 μmoles of glutamate (BDH), 100 μmoles of phosphate buffer (pH 7.4), 4 μmoles of INT and 0.1 μmoles of NAD (E. Merck) in a final volume of 3 ml. The reduced formazon was extracted with 5 ml of toluene and the optical density was measured at 495 μm with Hilger & Watts U. V. Speck. Protein levels were estimated by the method of Lowry *et al.* (1951). The

hepatopancreas isolated for the determination of water content was blotted gently on Watmann No. 1 filter paper and weighed. The tissues were kept in a hot-air oven at 100° C for 48 hours and the dry weight was determined.

RESULTS AND DISCUSSION

MEENAKSHI (1956a, 1964) reported no significant changes in the water content of the tissues in active and 6-months aestivated snails and these results agree with the present findings (Figure 1). MEENAKSHI (1956b) reported protein utilization in the aestivated snail, *Pila virens*. The protein levels in our findings have shown a decrease from the second month of aestivation, agreeing with the results of Meenakshi (Figure 2).

Changes in the enzyme activities in general during aestivation and hibernation in molluscs were reported (BALDWIN, 1938; CZAPSKA, 1959; ECKSTEIN & ABRAHAM, 1959; CHAFFE *et al.*, 1961; ANJANIPRASAD & KRISHNAMURTHY, 1962; BRYANT *et al.*, 1964; R. REDDY, 1964, 1965; MOHAN & DASS, 1969; RAO, 1973; S. REDDY, 1973). R. REDDY (1965) has reported a general decrease in the activity of glutamate dehydrogenase along with the alcohol and succinate dehydrogenases and other respiratory

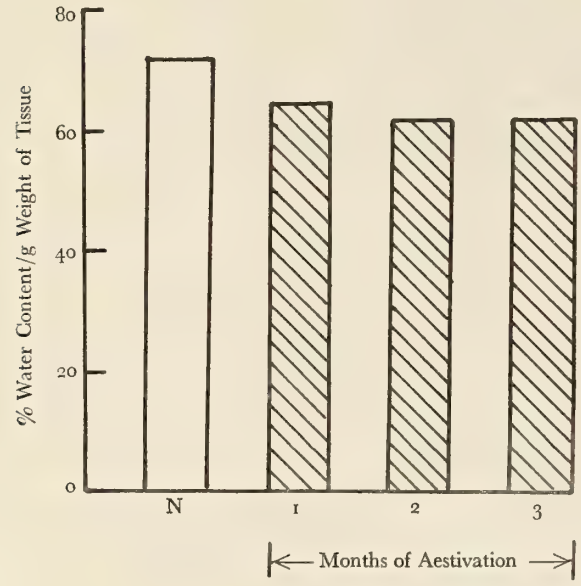

Figure 1

Water Content in the Normal and Aestivated *Pila globosa*
(lined histograms represent the aestivated condition)

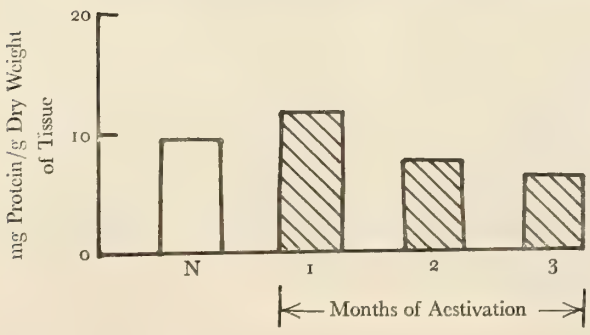

Figure 2

Protein Content in the Normal and Aestivated *Pila globosa*
(lined histograms represent the aestivated condition)

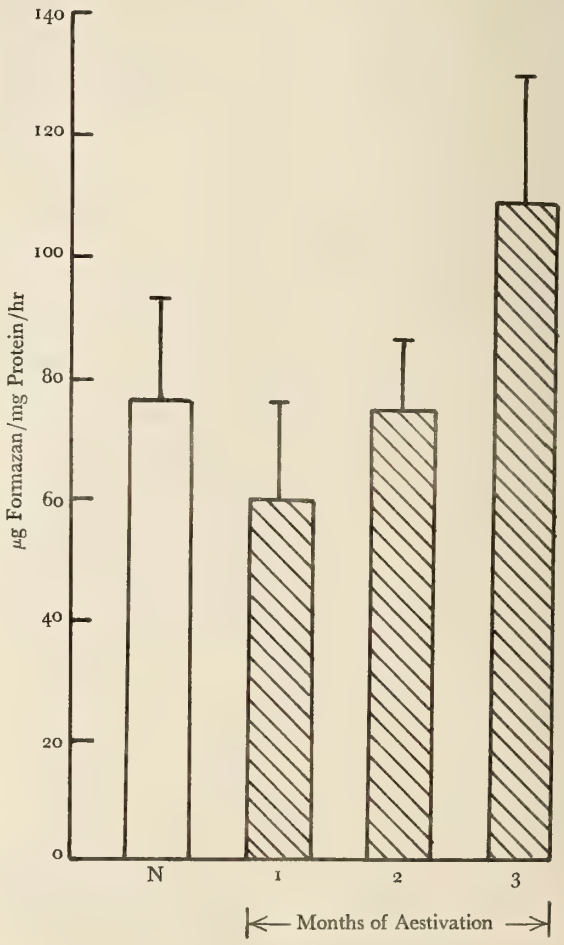

Figure 3

Glutamate Dehydrogenase Activity in the Normal and Aestivated *Pila globosa*
(lined histograms represent the aestivated condition)

enzymes during aestivation. In contrast to the above findings, it has been observed in the present investigation that glutamate dehydrogenase shows a peculiar behaviour during aestivation. The activity levels decreased in the first month of aestivation which is in agreement with the results of R. REDDY (1965). But with prolonged periods of aestivation the activity showed a general increase over the normal level (Figure 3).

MEENAKSHI (1965b) reported protein utilization in the aestivated snail *Pila virens* and protein utilization normally leads to an increased ammonia level in the organism (FRUTON & SIMMONDS, 1960; HARPER, 1971). NAYEEMUNNISA (1972) reported decreased ammonia content in the mantle during aestivation. Results of S. REDDY (1973) were also in agreement with the results of Nayeemunnisa for the mantle, but in other tissues (foot, hepatopancreas and body fluids) the ammonia levels were constant during aestivation. Further, the works of the above cited authors, and of LAL & SAXENA (1952) and R. REDDY (1963) have shown increased uric acid content during aestivation in the soft parts of *Pila globosa*. The levels of aspartic acid and glutamic acid were found to increase in different tissues of the aestivating *P. globosa* (MOHAN, 1973; RAO, 1973). Under these physiological circumstances the glutamate dehydrogenase is expected to increase in the aestivation, as discussed below.

With the rise in the glutamate dehydrogenase activity, the ammonia produced by the protein utilization reacts with the endogenous α-ketoglutarate to form glutamate (HARPER, 1971). The glutamate thus formed may be metabolised in 2 ways, *viz.*, (1) by transamination and (2) by amidation. By transamination with oxaloacetic acid, aspartate is formed (MULLER & LEUTHARDT, 1950; HARPER, 1971; MAHLER & CORDES, 1969). The aspartate thus formed would be the source for nitrogen 1 of the purine ring and fumaric acid is the by-product (HARPER, *op. cit.*; LONG, 1961; MAHLER & CORDES, *op. cit.*). Fumaric acid is converted into oxaloacetic acid through the Krebs cycle enzymes (HARPER, *op. cit.*; LONG, *op. cit.*; MAHLER & CORDES, *op. cit.*). During transamination it is interesting to observe that α-ketoglutarate is also formed (MULLER & LEUTHARDT, *op. cit.*) Thus, it is likely that this regeneration of α-ketoglutarate and oxaloacetic acid may act as a compensatory mechanism and as a contributory factor for their maintenance at a constant level during aestivation, wherein the oxidative metabolism is avoided in favour of glycolysis (MEENAKSHI, 1956a). The second path for the glutamate utilization is by amidation to glutamine with ammonia (MAHLER & CORDES, *op. cit.*; HARPER, *op. cit.*), which supplies nitrogen atoms 3 and 9 of the purine ring and the by-product being glutamic acid (LONG, *op. cit.*; MAHLER & CORDES, *op. cit.*; HARPER, *op. cit.*). This may be the reason for the increased glutamate content of the hepatopancreas in the aestivating snail. Hepatopancreas being the site for uric acid biosynthesis (LAL & SAXENA, 1952), increased xanthine dehydrogenase activity was also reported during aestivation (S. REDDY, 1973). The aspartate and glutamate thus formed may constitute the precursors for the uric acid biosynthesis. Further investigations are being carried out in this laboratory to ascertain the exact role of aspartic and glutamic acids in the aestivation metabolism of *Pila globosa*.

SUMMARY

1. Water content, protein levels and levels of glutamate dehydrogenase activity were estimated in the hepatopancreas of normal and aestivated *Pila globosa*.
2. No significant loss of water content was observed. Protein levels were found to decrease.
3. Glutamate dehydrogenase activity was observed to increase in the aestivated *Pila globosa*, except in the first month.
4. These results were correlated with the increased uric acid biosynthesis.

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